NeuroImage: Clinical 18 (2018) 694–701

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Altered network hub connectivity after acute LSD administration

Felix Müller[a], Patrick C. Dolder[b], André Schmidt[a], Matthias E. Liechti[b], Stefan Borgwardt[a,*]

[a] University of Basel, Department of Psychiatry (UPK), Basel 4012, Switzerland
[b] University of Basel, Division of Clinical Pharmacology and Toxicology, Department of Biomedicine and Department of Clinical Research, University Hospital Basel, Basel 4031, Switzerland

ARTICLE INFO

Keywords:
LSD
fMRI
Functional connectivity
Networks
Hubs

ABSTRACT

LSD is an ambiguous substance, said to mimic psychosis and to improve mental health in people suffering from anxiety and depression. Little is known about the neuronal correlates of altered states of consciousness induced by this substance. Limited previous studies indicated profound changes in functional connectivity of resting state networks after the administration of LSD. The current investigation attempts to replicate and extend those findings in an independent sample. In a double-blind, randomized, cross-over study, 100 µg LSD and placebo were orally administered to 20 healthy participants. Resting state brain activity was assessed by functional magnetic resonance imaging. *Within*-network and *between*-network connectivity measures of ten established resting state networks were compared between drug conditions. Complementary analysis were conducted using resting state networks as sources in seed-to-voxel analyses. Acute LSD administration significantly decreased functional connectivity *within* visual, sensorimotor and auditory networks and the default mode network. While *between*-network connectivity was widely increased and all investigated networks were affected to some extent, seed-to-voxel analyses consistently indicated increased connectivity between networks and subcortical (thalamus, striatum) and cortical (precuneus, anterior cingulate cortex) hub structures. These latter observations are consistent with findings on the importance of hubs in psychopathological states, especially in psychosis, and could underlay therapeutic effects of hallucinogens as proposed by a recent model.

1. Introduction

In recent years, there has been increasing interest in research on hallucinogenic drugs such as psilocybin, DMT (*N*,*N*-dimethyltryptamine), and LSD (lysergic acid diethylamide) (Nichols, 2016; Liechti, 2017). Several effects induced by hallucinogenic drugs resemble symptoms observed in schizophrenia (Gouzoulis-Mayfrank et al., 1998) and these drugs are therefore thought to serve as models of psychosis and might provide further insight into the pathophysiology of this mental disease (Geyer and Vollenweider, 2008). It is also assumed, that hallucinogens can improve mental health in people suffering from anxiety and depression (Nichols, 2016; Nichols et al., 2016). Compared with conventional psychopharmacological treatments, this approach is promising because long-lasting improvements resulting from one or a few administrations have been reported (Nichols et al., 2016). However, little is known about the neural correlates underlying these effects. Functional magnetic resonance imaging (fMRI) can be used to assess hemodynamic signatures as an indirect measure for neuronal activity. Two recent articles (Carhart-Harris et al., 2016b; Tagliazucchi et al., 2016) on the neuronal effects of LSD reported widespread alterations in functional connectivity (FC), a measure used to characterize functional integration by assessing the statistical dependency between spatially remote brain regions (Friston, 2011). It was found that LSD acutely *decreased* coactivation *within* several resting state networks (RSNs), but *increased* FC *between* networks (Carhart-Harris et al., 2016b). The same group also reported *increases* in between network FC after the administration of the related substance psilocybin in another sample (Carhart-Harris et al., 2013; Roseman et al., 2014). However, those studies were limited by small sample sizes and significant differences in head motion between conditions, which could have biased the results (Power et al., 2014). Apart from one report on DMT, which confirmed decreased FC within the default mode network (DMN) (Palhano-Fontes et al., 2015), no attempt has been made to replicate these findings in an independent sample. Moreover, the significance of these findings with regard to subjective drug effects is largely unknown (Carhart-Harris et al., 2016b).

In this placebo-controlled study, we investigated the effect of 100 µg LSD on RSN FC in 20 healthy subjects using fMRI. Based on the identification of ten well established RSNs (Smith et al., 2009) and following the approaches of studies described above (Carhart-Harris et al.,

* Corresponding author at: University of Basel, Department of Psychiatry (UPK), Wilhelm Klein-Strasse 27, Basel, Switzerland.
E-mail address: stefan.borgwardt@upkbs.ch (S. Borgwardt).

https://doi.org/10.1016/j.nicl.2018.03.005
Received 3 August 2017; Received in revised form 15 January 2018; Accepted 6 March 2018
Available online 07 March 2018

2013; Roseman et al., 2014; Carhart-Harris et al., 2016b), the effects of LSD on FC within and between those networks were assessed in an attempt to replicate previous findings (Carhart-Harris et al., 2016b; Carhart-Harris et al., 2013; Roseman et al., 2014). RSNs were identified using independent component analysis (ICA), a widely used, data-driven method to identify spatially distributed signal components in fMRI data (Beckmann et al., 2009). With this approach, coherent RSNs can be identified and then further analyzed. RSNs can be subdivided into the default mode network (DMN) and other RSNs. The DMN is particularly activated during rest and is thought to be involved in functions such as self-referential thinking (Buckner et al., 2008; Raichle et al., 2001). On the contrary, other RSNs observed during rest closely resemble networks involved in demands across different tasks (Smith et al., 2009).

As the data on associations between the described FC measures and subjective drug effects are limited, we also conducted an exploratory correlation analysis. Additionally, we tested the only pre-described associations between decreased FC within the DMN and the subjective experience of "ego dissolution" (Carhart-Harris et al., 2016b). Furthermore, seed-to-voxel analyses were performed to characterize changes in the connectivity of RSNs to other regions on a whole-brain level in more detail – concentrating on changes in FC between RSNs and hub structures. Hubs are thought to serve integration and large-scale interactions (van den Heuvel and Sporns, 2013; Bell and Shine, 2016) and have been implicated as critical structures in various mental diseases, including psychosis (Crossley et al., 2014). These structures are therefore likely to be involved in hallucinogen-induced alterations in network FC.

2. Materials and methods

This study was a randomized, placebo-controlled, double-blind, cross-over trial. Each participant received 100 μg LSD and placebo in a cross-over manner. The washout period between sessions was at least seven days. Functional MRI data was acquired for each condition, resulting in two data sets (active drug and placebo) for each participant (repeated measures design). Conditions were compared using paired *t*-tests. The study was approved by the Ethics Committee for Northwest/Central Switzerland (EKNZ) and by the Federal Office of Public Health. All subjects provided written consent prior to participating and received monetary compensation. This study was registered at clinicaltrials.gov prior to study start (NCT02308969).

2.1. Subjects

Participants were recruited by advertisement and word of mouth. All participants were screened by general medical examination and psychiatric interview to assess the following exclusion criteria: age < 25 or > 65 years, personal or first degree relative with an axis I major psychiatric disorder, history of drug dependence, cardiac or neurological disorders, use of any regular medication pregnancy, nursing, hypertension (> 140/90 mm Hg) or hypotension (SBP < 85 mm Hg), smoking > 10 cigarettes/day, use of illicit drugs (except tetrahydrocannabinol) > 10 times or any time within the previous two months (as assessed by history and urine tests). Twenty healthy subjects out of initially 24 participants were included (10 male, 10 female; mean age 32 ± 11 years; range: 25–60 years, all but one with university education and all right-handed). Data sets from four subjects were excluded from analysis due to movement during the fMRI scan (see below). Only two participants had used a hallucinogenic drug before, both on only a single occasion. For data on lifetime drug use of the 20 included subjects see Supplemental Table 1.

2.2. Assessments of plasma levels and subjective drug effects

Subjective effects were assessed three hours after administration of placebo or LSD (directly after the MRI scan), using the 5 dimensions of altered states of consciousness (5D-ASC) scale (Dittrich, 1998). This well validated questionnaire (Studerus et al., 2010) was designed to measure altered states of consciousness via visual-analog scales. Venous blood samples were collected in lithium heparin tubes two and three hours after administration, centrifuged and stored at −20 °C. Plasma LSD concentrations were measured using a validated tandem mass spectrometry method (Dolder et al., 2017a).

2.3. Assessment of blood pressure, heart rate, and body temperature

Blood pressure, heart rate, and body temperature were assessed two and three hours after drug administration. Blood pressure and heart rate were measured twice at both time points. The average of both measurements was used for further analysis.

2.4. Image acquisition

Images were acquired using a 3 Tesla MRI system (Magnetom Prisma, Siemens Healthcare, Erlangen, Germany) with a 20-channel phased array radio frequency head coil. Anatomical images were acquired using a T_1-weighted magnetization prepared rapid acquisition gradient (MPRAGE) sequence (field of view: 256 × 256 mm^2; resolution: 1 × 1 × 1 mm^3; repetition time: 2000 ms; echo time: 3.37 ms; flip angle: 8°; bandwidth: 200 Hz/pixel). This was followed by an interleaved T2*-weighted echo planar imaging resting state sequence. The following parameters were used: 35 axial slices with a slice thickness of 3.5 mm, a 0.5 mm inter-slice gap, a field-of-view of 224 × 224 cm^2, and a resolution of 3.5 × 3.5 × 3.5 mm^3. The repetition time was 1.8 s, echo time 28 ms, *flip angle 82*°, and bandwidth 2442 Hz/pixel. During the scan, participants were instructed to close their eyes and remain awake. Three hundred volumes were acquired. This resting state scan was followed by other sequences not relevant for this publication. Total acquisition time was approximately 50 min. Three hundred volumes were acquired. Subjects with head motion of > 2 mm translation or > 2° rotation were excluded (n = 4), resulting in a sample of 20 subjects. No significant differences in head motion were present between conditions (see Supplementary for more details).

2.5. Preprocessing

Preprocessing was performed using FEAT (FMRI Expert Analysis Tool) Version 6.00 (part of FSL, www.fmrib.ox.ac.uk/fsl) and SPM12 (http://www.fil.ion.ucl.ac.uk/spm/). Five dummy scans were excluded and the remaining volumes were quality checked for head motion and image artifacts. Preprocessing for all volumes included brain extraction, realignment to the first volume, slice time correction, co-registration to the T_1-weighted structural volume, segmentation, normalization into a standard stereotactic space (Montreal Neurological Institute) and smoothing with a 5 mm full width at half maximum Gaussian kernel. Preprocessing for the ICA analysis included masking of non-brain voxels, highpass temporal filtering (0.01 Hz cut-off), voxel-wise demeaning of the data and normalization of the voxel-wise variance. Data were whitened and projected into a 20-dimensional subspace using Principal Component Analysis. Preprocessing of between-network connectivity and seed-to-voxel analysis included scrubbing (global signal threshold of z > 3, composite subject motion threshold of > 0.5 mm) using ART as implemented in CONN and linear regression of the six motion parameters and the effects of each condition. Five principal components were extracted from white matter and cerebrospinal fluid signals (using individual tissue masks obtained from the T_1-weighted structural images) and removed using CompCor (Behzadi et al., 2007). These components are thought to reflect noise (especially motion and physiological fluctuations) and are therefore removed from the time series. The resulting functional images were band-pass filtered (0.008 < f < 0.09 Hz).

2.6. Independent component analysis

ICA was performed using Probabilistic Independent Component Analysis as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version 3.14 (part of FSL, www.fmrib.ox.ac.uk/fsl). This study was restricted to 20 components, in order to allow direct comparison with the templates used by Smith et al. (2009) and previous studies using LSD (Carhart-Harris et al., 2016b) and psilocybin (Carhart-Harris et al., 2013). The resulting 20 components were visually inspected and components which largely reflected artifacts (8 components) were removed before further analysis using previously described procedures (Kelly Jr. et al., 2010). The remaining 12 components were matched with the ten primary resting state reference networks via visual inspection and cross-correlation, as described by Smith et al. (2009). These 10 networks identified in our data set were then used for all further analyses.

2.7. Within-network connectivity

Subject-specific versions of the 10 RSNs in our data set were generated using dual regression (Beckmann et al., 2009). Differences between the LSD and the placebo condition in each component were assessed by a permutation test using Randomise in FSL (paired two-tailed *t*-test, 5000 permutations). Clusters were formed using threshold-free cluster enhancement. To account for testing 10 networks, the initial threshold of $p < 0.05$ (FWE) was adjusted (Bonferroni correction), resulting in a threshold of $p < 0.005$ (FWE).

2.8. Between-network connectivity and seed-to-voxel analysis

Analysis of FC between RSNs and seed-to-voxel analysis were performed using the CONN functional connectivity toolbox 17a (http://www.nitrc.org/projects/conn) (Whitfield-Gabrieli and Nieto-Castanon, 2012). Unthresholded maps of RSNs obtained from ICA analysis were imported as ROIs and the weighted sums of the time series were extracted. For analysis of FC between networks, time courses between all ten RSNs were compared for both conditions using bivariate correlation. The resulting correlation coefficients were compared between placebo and drug conditions, using paired *t*-tests (two tailed). Results were corrected for multiple comparisons (10 × 10 networks) using the false discovery rate (FDR). Significance for FDR was assumed at $p < 0.05$. For seed-to-voxel analysis, the same ROIs were used. Time courses of each RSN were correlated with time courses of each voxel throughout the brain. Differences between placebo and drug condition were assessed using paired *t*-tests (two tailed). Results for FDR were considered significant at a cluster threshold of $p < 0.005$ (two tailed) on the basis of a cluster-forming threshold of $p < 0.001$.

2.9. Relationship with subjective drug effects and plasma levels

Associations between subjective drug effects and alterations of within-network FC were tested in an exploratory correlations analysis. Individual parameter estimates (PE) obtained from within-network connectivity analysis (ΔPE = mean PE_{LSD} − mean $PE_{Placebo}$) were extracted from significantly altered clusters within RSNs ($p < 0.005$, FWE, threshold-free cluster enhancement) and then correlated (Pearson's r) with subjective ratings in the 5D-ASC total scores and in all five major dimensions of the 5D-ASC. We further tested the association between decreased FC within the DMN and subjective ratings on the 5D-ASC score “ego dissolution” (item 71 of the questionnaire), as previously reported (Carhart-Harris et al., 2016b). No other significant associations have been reported. Correlations with the plasma levels of LSD were calculated using the individual plasma levels of LSD measured at 2 h after administration – directly before the MRI scan, as the resting state sequence was the first sequence in our protocol. Calculations were performed using SPSS version 23.00 (IBM).

2.10. Relationship with blood pressure, heart rate, and body temperature

It has already been shown that LSD increases physiological parameters (PP) such as blood pressure, heart rate and body temperature (Schmid et al., 2015; Dolder et al., 2017b). As all of these factors might bias FC results (Khalili-Mahani et al., 2013), a second correlation analysis was performed in order to assess possible associations. This analysis was conducted in the same manner as described above, i.e. alterations in physiological parameters ($\Delta PP = PP_{LSD} - PP_{Placebo}$) at 2 h after administration were correlated (Pearson's r) with individual FC measures ($\Delta PE = PE_{LSD} - PE_{Placebo}$) of significant FC results. This was done for systolic blood pressure (ΔSBP), diastolic blood pressure (ΔDBP), heart rate (ΔHR), and body temperature (ΔBT) and significant FC results of all three analysis (within network FC, between network FC and seed-to-voxel analysis). Results were corrected for multiple comparisons (FDR).

Please see online Supplement for additional methods detail.

3. Results

3.1. Identification of resting state networks

Of the 20 components of the ICA analysis, eight were classified as mainly reflecting artifacts. The remaining 12 components were cross-correlated to the ten templates provided by Smith et al. (2009). All ten templates were identified in our data set and were labeled accordingly (see Supplemental Fig. 2): Visual networks 1–3 ($r = 0.87$, $r = 0.35$, $r = 0.64$), DMN ($r = 0.82$), cerebellum ($r = 0.62$), sensorimotor network ($r = 0.79$), auditory network ($r = 0.70$), executive control network ($r = 0.55$), and frontoparietal networks 1–2 ($r = 0.57$, $r = 0.53$). Two networks corresponded to RSNs not included in the ten templates.

3.2. Within-network connectivity

The drug and placebo conditions were compared between all ten networks. Significantly decreased coactivation under the drug condition relative to placebo was found in visual networks 1 and 3 (covering almost all parts of these networks), in large parts of the sensorimotor network, in the medial-posterior part of the DMN, and a small cluster in the right inferior frontal gyrus of the auditory network. Results are shown in Fig. 1 and online Supplemental Table 2. We observed increased coactivation relative to placebo in small clusters in areas that were not covered by the networks in the analysis of the visual networks 2 (right superior parietal lobe) and 3 (precuneus), and of the frontoparietal network 1 (left inferior frontal gyrus; see Supplemental Fig. 3).

3.3. Between-network connectivity

Widespread increases in between-network FC were observed under LSD compared with placebo. All investigated networks were affected to some extent; this was most pronounced for the visual network 1, the cerebellum and the executive network (see Fig. 2). No decreases in FC were observed.

3.4. Seed-to-voxel analysis

Seed-to-voxel analysis using all ten networks as seeds also reflected profound increases in FC (see Supplemental Fig. 4). Visual inspection of the results indicated that certain regions were repeatedly involved (especially occipital and subcortical areas). This finding was further investigated by merging all results shown in Supplemental Fig. 4 to illustrate overlaps (see Fig. 3A). Overlaps of more than four components were observed in the cerebellum, occipital regions (lingual gyrus, intracalcarine cortex, precuneus), frontal regions (frontal medial cortex, right frontal pole, anterior cingulate cortex), and subcortical structures (bilateral thalamus, putamen, pallidum, and caudate). The largest

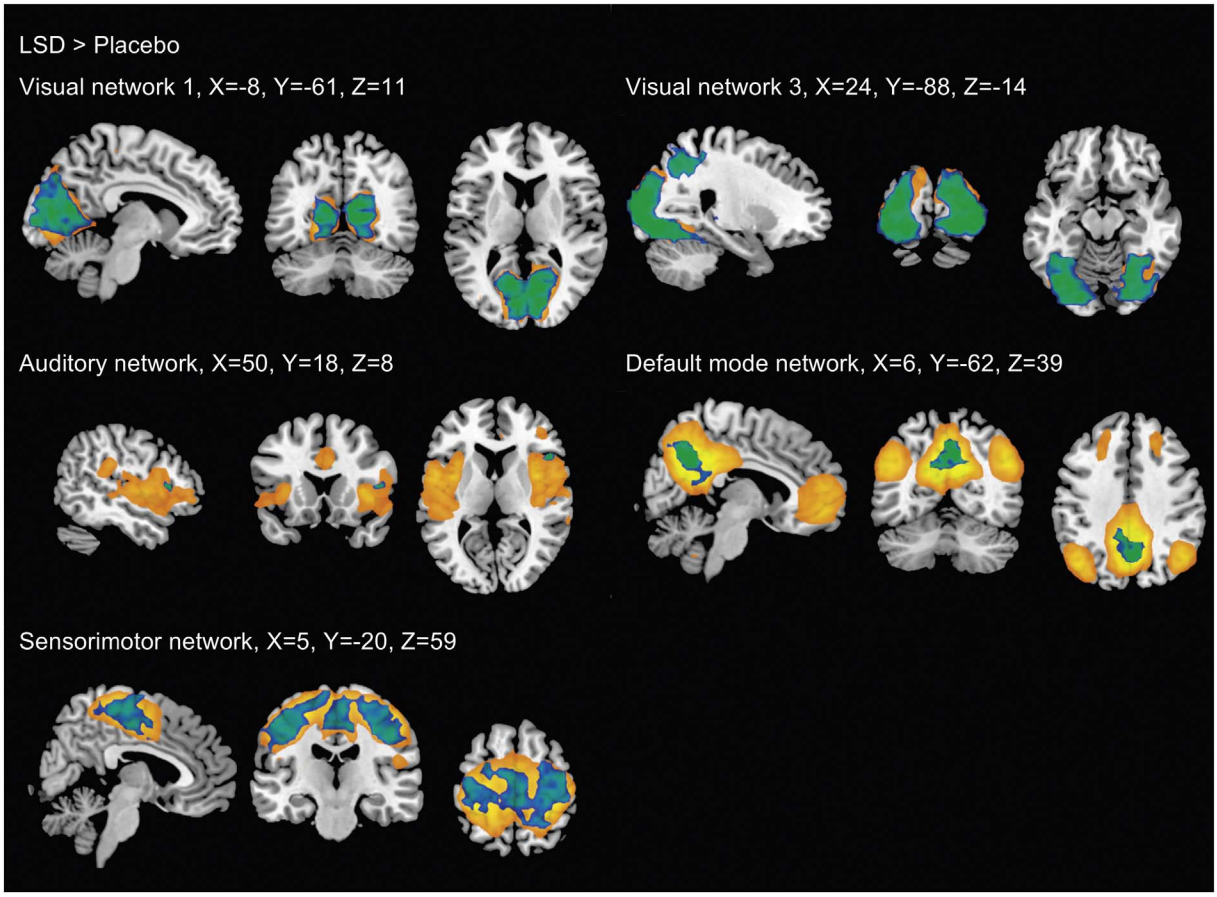

Fig. 1. Between-condition differences in within-network functional connectivity. Only decreased connectivity (shown in blue-green) was observed for the contrast LSD > placebo. Results are overlayed on the corresponding resting state networks obtained in our analysis (yellow). Images are thresholded at $p < 0.005$ (two tailed), FWE (threshold free cluster enhancement). X, Y, and Z values indicate MNI coordinates. Right is the right side of the brain. (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this chapter.)

overlaps (> 6 components) were observed in the bilateral thalamus and caudate. Detailed projections of RSNs on these structures are shown in Fig. 4.

3.5. Analysis of hub structures

As several of these regions are known hubs and are thus thought to serve integration and large-scale interaction between brain regions, we suspected that these structures might be critically involved in drug-induced changes in large-scale FC. To investigate this possibility, the main effect of all networks was assessed by simultaneously including all ten networks as seeds and calculating a weighted contrast (reflecting the common effect of these seeds on voxels covering the whole brain; cluster threshold $p < 0.05$, FDR, cluster-forming threshold $p < 0.001$ uncorrected). Common effects were observed in a few regions (see Fig. 3B and Supplemental Table 3), particularly subcortical structures (thalamus, putamen, caudate, cerebellum), and precuneus and frontal areas (anterior cingulate cortex, frontal pole, middle frontal gyrus).

3.6. Relationship with subjective drug effects and plasma levels

Decreased FC within the auditory network correlated significantly with the 5D-ASC major dimensions “visionary restructuralization” ($r = -0.53$, $p = 0.02$), and “auditory alterations” ($r = -0.49$, $p = 0.03$). However, these associations did not remain significant when adjusted for multiple comparisons (FDR). No other significant correlations between within-network FC and subjective effects were observed (see Supplemental Table 4). No significant associations between FC measures and plasma levels were observed (see Supplemental Table 4). No significant correlation was observed between DMN integrity and subjective ratings on the item “ego dissolution” ($r = 0.06$, $p = 0.81$).

3.7. Relationship with blood pressure, heart rate, and body temperature

No significant correlations ($p < 0.05$) between physiological parameters and FC measures were observed for within-network connectivity and for seed-to-voxel analysis. For between-network connectivity, there were some significant correlations between ΔSBP, ΔDBP, ΔBT and FC measures (ΔSBP and visual network 1-visual network 3: $r = 0.64$, $p < 0.01$; ΔSBP and sensorimotor network – executive network: $r = -0.54$, $p = 0.01$; ΔDBP and visual network 1 – visual network 3: $r = 0.52$, $p = 0.01$; ΔBT and visual network 2 – executive network: $r = 0.46$, $p = 0.04$). However, none of these results remained significant after correction for multiple comparisons (FDR). Even more importantly, there was no evidence for a systematic (non-significant) relationship between one of the physiological parameters and alterations in FC induced by LSD. All results are presented in Supplemental Fig. 5. Additionally, physiological parameters under the placebo and the drug condition are given in Supplemental Table 5.

4. Discussion

This study investigated the acute effects of LSD on network connectivity in healthy subjects. We found that acute LSD administration decreased FC *within* the DMN, the auditory and sensorimotor networks and visual networks 1 and 3. *Between*-network FC was widely increased;

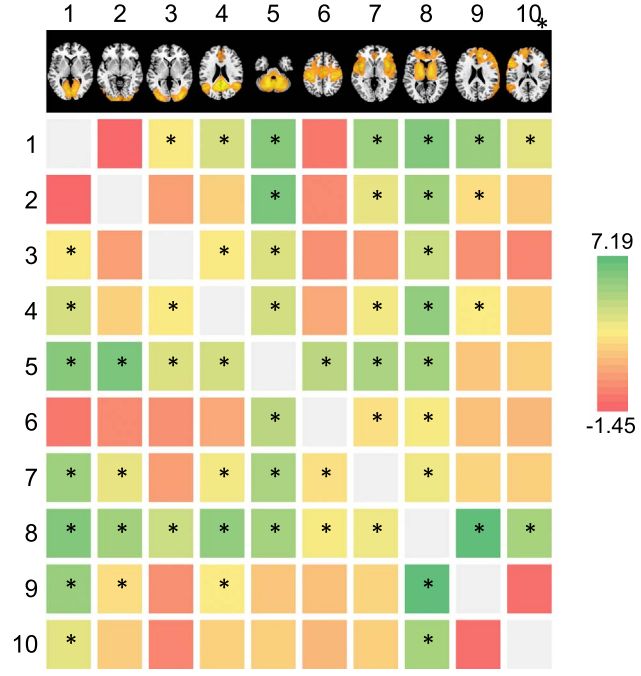

Fig. 2. Correlation matrix illustrating widespread increased functional connectivity between resting state networks under the drug condition compared to placebo. The colorbar indicates the t value, asterisks indicate significant differences ($p < 0.05$, FDR, two tailed). (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this chapter.)

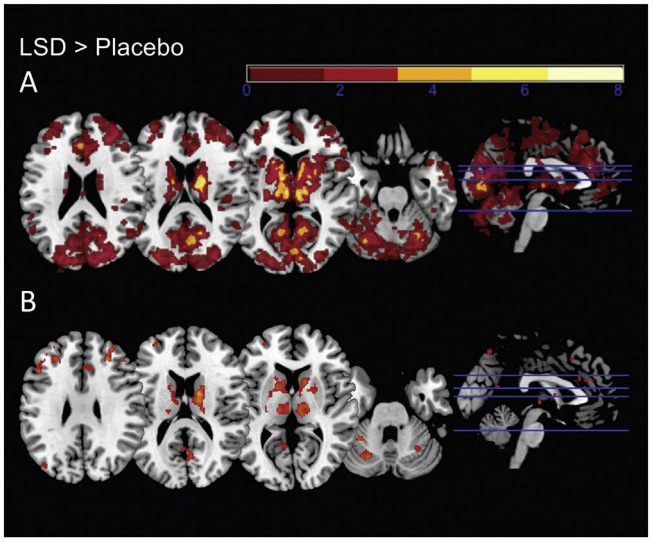

Fig. 3. A: Results of seed-to-voxel analysis shown in Supplemental Fig. 4 combined in one map. The color bar indicates the number of converging components. B: Main effect of all ten resting state networks in seed-to-voxel analysis (cluster threshold $p < 0.05$, FDR, two tailed, cluster-forming threshold $p < 0.001$ uncorrected). Right is right side of the brain. (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this chapter.)

all RSNs were affected to some degree, particularly the cerebellum and the executive network. Seed-to-voxel analysis indicated that increased FC of RSNs on a whole-brain level consistently involved hub structures (specifically thalamus and striatum). Below, we discuss our findings in the context of previous observations obtained after the administration of LSD (Carhart-Harris et al., 2016b; Tagliazucchi et al., 2016) and the related hallucinogenic drug psilocybin (Carhart-Harris et al., 2013; Roseman et al., 2014). Like LSD, psilocybin exerts its effects mainly through agonism at the 5-HT_{2A}-receptor (Vollenweider et al., 1998; Preller et al., 2017).

Our results on alterations in *within*-network connectivity closely resemble those previously reported in LSD (Carhart-Harris et al., 2016b), where decreases in coactivation within the DMN, the sensorimotor network and the visual networks 1 and 3 were reported. Additionally, Carhart-Harris et al. reported decreases in the right frontoparietal network and the parietal cortex network and found no alterations within the auditory network. We did not investigate the parietal network in our study and the frontoparietal network was not affected in our analysis. The functional relevance of decreased within-network FC to subjective drug effects is largely unknown. No significant associations were described in previous studies with psilocybin (Carhart-Harris et al., 2013; Roseman et al., 2014). For LSD, decreased coactivation within the DMN was significantly associated with feelings of "ego dissolution" (Carhart-Harris et al., 2016b), a finding that could not be replicated in our study – although ego dissolution varied markedly across subjects and correlated with exposure to LSD (Liechti et al., 2016). Within this context, it should be noted, that very similar alterations in within-network connectivity were reported after the administration of sertraline, a selective serotonin reuptake inhibitor (Klaassens et al., 2015). Klaassens et al. studied the effects of sertraline on connectivity using the same RSN templates and similar methods of analysis; they pinpointed exactly the same networks as in our study (DMN, visual networks 1–3, sensorimotor and auditory network). These findings also overlapped with regions identified in a previous study with LSD (Carhart-Harris et al., 2016b). These effects of sertraline, a drug causing no major subjective effects, call into question the specificity of these alterations and their significance for subjective drug effects. It is possible that they represent an epiphenomen of unspecific serotonergic stimulation.

Between-network connectivity was examined in two previous studies after administration of either psilocybin (Carhart-Harris et al., 2013; Roseman et al., 2014) or LSD (Carhart-Harris et al., 2016b). With psilocybin, FC between an anterior DMN and nine other RSNs was investigated (Carhart-Harris et al., 2013); FC was increased between the anterior DMN and dorsal attention, salience, right frontoparietal, and auditory networks. Broader analyses of FC between several networks indicated widespread increases in between-network FC under psilocybin (Roseman et al., 2014) and – to a considerably lesser extent – under LSD (Carhart-Harris et al., 2016b). Fig. 5 shows a comparison of these findings of these studies with our results. In general, there was no good accordance within the results of previous studies (Roseman et al., 2014; Carhart-Harris et al., 2016b) or between those studies and our findings. It does not seem very likely that these widely divergent findings simply reflect different effects of psilocybin and LSD, as their mechanism of action at the $5HT_{2A}$-receptor is very similar (Rickli et al., 2016), as are probably the subjective effects (Hollister and Hartman, 1962; Hollister and Sjoberg, 1964). Furthermore, our results differed considerably from those recently reported for LSD (Carhart-Harris et al., 2016b). These differences might be due to significant differences in movement between conditions present in those studies and different methods of analysis, differences in regions covered by RSNs, or the slightly larger sample size in our study. Moreover, routes of administration (i.v. and oral administration) or the previous experiences of participants with hallucinogenic drugs might have affected the results.

Seed-to-voxel analyses indicated increased FC between RSNs and several structures known as hubs (precuneus, anterior cingulate cortex, striatum and thalamus). Hubs are thought to be of importance for overall brain functioning by serving integration and large-scale interaction (van den Heuvel and Sporns, 2013; Bell and Shine, 2016). Using functional connectivity density analysis, a previous study has already reported significantly increased global connectivity in several regions after the administration of LSD and psilocybin, including the precuneus and thalamus (Tagliazucchi et al., 2016). In the present study, it was found that FC between the great majority of RSNs and striatum and thalamus was increased after administration of LSD, compared with

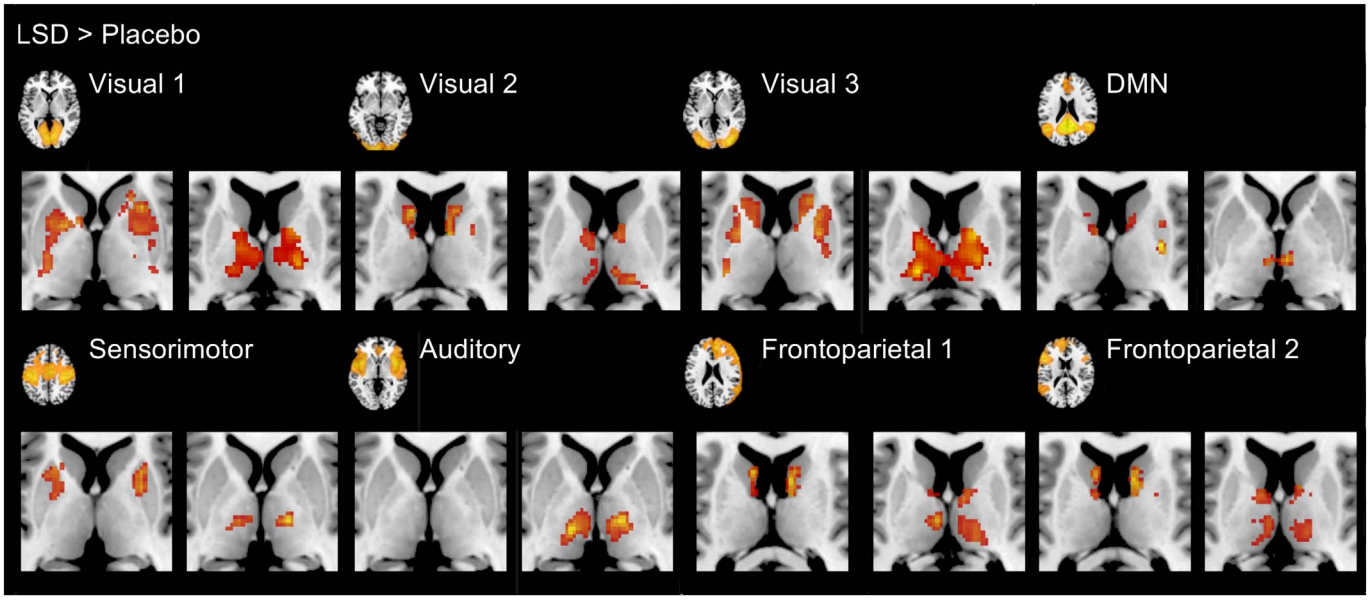

Fig. 4. Functional projections of resting state networks on striatal (left) and thalamic (right) structures. Only increases in FC were observed (cluster threshold $p < 0.005$, FDR, two tailed, cluster-forming threshold $p < 0.001$). Right is right side of the brain.

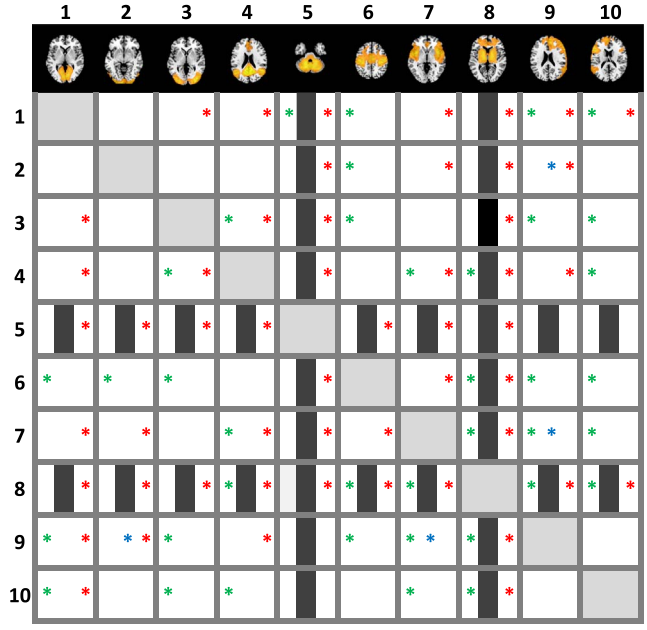

Fig. 5. Comparison of the effects of hallucinogenic drugs on between-network connectivity obtained by another group (Carhart-Harris et al., 2016b; Roseman et al., 2014) and our results. All results on networks identified in the present study were included. Asterisks indicate significant differences between drug and placebo condition (green: Roseman et al., 2014; blue: Carhart-Harris et al., 2016b; red: present study). Black indicates networks which were not investigated. Roseman et al. reported separate results for two representation of the DMN, which were combined in this presentation. (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this chapter.)

placebo. Both structures (striatum and thalamus) show widespread structural connections to other brain regions (Jones, 2007; Parent and Hazrati, 1995) and are part of the so called "rich club", a set of highly connected regions that are densely connected among themselves (van den Heuvel and Sporns, 2011; Schmidt et al., 2016). The striatum (comprised of caudate and putamen) is the main input structure of the basal ganglia (Parent and Hazrati, 1995) and is connected to numerous cortical regions via loops (Alexander et al., 1986) which pass through the basal ganglia, to the thalamus and back to the cortex (cortico-striato-thalamo-cortical loops). We have already investigated thalamocortical FC after administration of LSD in the same sample in more detail (Müller et al., 2017). Among others, we found increased global FC of striato-thalamic structures after LSD compared with placebo. This could indicate that these regions are specifically influenced by LSD. It is interesting to note that the thalamic and striatal projections for the DMN, sensorimotor and frontoparietal networks observed in this study were relatively similar to the topography of cortical projections on these regions observed in several fMRI studies (Hale et al., 2015; Yuan et al., 2016; Zhang et al., 2008; Zhang et al., 2010; Choi et al., 2012). However, the visual networks 1–3 and the auditory network did not follow this pattern, and occupied large portions of these subcortical structures.

The importance of hub lesions in brain disorders has recently been emphasized (Crossley et al., 2014). Alterations in thalamocortical FC have long been suspected to be involved in the pathophysiology of schizophrenia (Lisman et al., 2010; Pinault, 2011; Ferrarelli and Tononi, 2011) and have been one of the few neuroimaging findings that have been repeatedly replicated in the search of the neural correlates of schizophrenia. Several studies indicated that FC between thalamus and frontal regions is decreased while FC between thalamus and sensorimotor areas is increased (for an overview see (Giraldo-Chica and Woodward, 2017)). It is remarkable that data-driven FC analysis in one of the biggest samples to date has identified alterations in thalamocortical FC as an outstanding characteristic of schizophrenia (Cheng et al., 2015). The findings reported by those authors very closely resemble those found in our data set (see Fig. 3) and, additionally to the thalamus, also comprise the pallidum and striatum. These observations might indicate that not only the thalamus but also the whole cortico-striato-thalamo-cortical circuitry is involved in altered brain functioning in schizophrenia as well as in altered states of consciousness induced by LSD, as has already suspected (Geyer and Vollenweider, 2008; Andreasen, 1999). Maybe these similarities could also explain why hallucinogenic drugs can induce psychotic episodes in vulnerable subjects (Vardy and Kay, 1983), rather than in the general population (Johansen and Krebs, 2015) as they might act on a system that is already impaired in patients in at-risk states of psychosis (Anticevic et al., 2015).

On the other side, a recent model proposed that altered hub connectivity induced by hallucinogens might also explain their therapeutic effects (Nichols et al., 2016). Various studies have indicated that these

substances might have beneficial effects in distinct mental disorders like depression, anxiety, and addiction. It is an interesting question, how a single mechanism of action can exert positive effects in heterogeneous diseases. Nichols et al. hypothesize that this link can be found in altered hub connectivity induced by these drugs. According to this model, pathological connectivity patterns associated with diverse mental diseases are acutely altered through destabilization of hub functions with subsequent changes in FC between various brain regions. These events somehow give rise to the development of new connectivity patterns which are stabilized after the acute effects have subsided, possibly through anti-inflammatory effects (Nichols et al., 2016).

This study is limited by a relatively small sample size and the use of a moderately high dose of LSD. Functional MRI is only an indirect measure for neuronal activity and relies on neurovascular coupling. LSD might alter neurovascular coupling, as already reported for the related serotonergic drug psilocin (Spain et al., 2015). This might result in biased FC results (Liu, 2013). LSD-induced alterations in physiological parameters such as heart rate and blood pressure might also affect FC (Khalili-Mahani et al., 2013). We found no evidence for a systematic relationship between alterations in physiological parameters and functional connectivity in our data set. However, these measures were taken only at one time point before the fMRI scan and nuisance regression of continuously recorded parameters might have been preferable.

Furthermore, the fMRI environment might have negatively influenced subjective drug effects (Studerus et al., 2012). Although hallucinogenic drugs seem to mimic some symptoms present in schizophrenia (Gouzoulis-Mayfrank et al., 1998; Carhart-Harris et al., 2016a; Liechti, 2017), there are presumably also important differences. Firstly, negative symptoms as commonly seen in schizophrenia are probably not present to this extent with hallucinogens, which mainly induce effects similar to positive symptoms (De Gregorio et al., 2016). Secondly, there are also important differences among the positive symptoms, such as the predominance of visual hallucinations in hallucinogens compared to mainly auditory hallucinations in schizophrenia. ICA results are influenced by the prespecified dimensionality (i.e. the prespecified number of components) (Ray et al., 2013). However, we restricted ICA to 20 components in order to allow direct comparison with established templates by Smith et al. (Smith et al., 2009) and with results from previous studies using LSD (Carhart-Harris et al., 2016b) and psilocybin (Carhart-Harris et al., 2013).

5. Conclusion

In the search of neuronal correlates of altered states of consciousness induced by LSD, we could replicate previous findings describing *decreased* functional connectivity *within* RSNs. However, the relevance and specificity of these alterations for LSD-induced effects are questioned by lacking associations with subjective drug effects as well as very similar observations obtained after the administration of a serotonin reuptake inhibitor. In line with previous results, we found widely *increased between*-network connectivity. Closer inspection, however, indicated very little consistencies in altered FC between specific RSNs in our sample and previous findings in LSD and psilocybin. Therefore, it seems doubtful that one of these measures is a reliable and characteristic neuronal correlate of hallucinogenic drug effects. Importantly, our results indicated increased connectivity between networks and subcortical and cortical hub structures. This finding is in line with previous observations in psychopathological states, especially in psychosis. According to a recent model, altered hub connectivity might also explain improvements observed in various mental diseases after the administration of hallucinogens.

Conflict of interest

None.

Funding

This work was supported by the Swiss National Science Foundation (grant number 320030_170249 to MEL and SB).

Acknowledgments

The authors would like to thank Dr. Sarah Longhi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2018.03.005.

References

Alexander, G.E., Delong, M.R., Strick, P.L., 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu. Rev. Neurosci. 9, 357–381.
Andreasen, N.C., 1999. A unitary model of schizophrenia: Bleuler's "fragmented phrene" as schizencephaly. Arch. Gen. Psychiatry 56, 781–787.
Anticevic, A., Haut, K., Murray, J.D., Repovs, G., Yang, G.J., Diehl, C., McEwen, S.C., Bearden, C.E., Addington, J., Goodyear, B., Cadenhead, K.S., Mirzakhanian, H., Cornblatt, B.A., Olvet, D., Mathalon, D.H., McGlashan, T.H., Perkins, D.O., Belger, A., Seidman, L.J., Tsuang, M.T., Van Erp, T.G., Walker, E.F., Hamann, S., Woods, S.W., Qiu, M., Cannon, T.D., 2015. Association of thalamic dysconnectivity and conversion to psychosis in youth and young adults at elevated clinical risk. JAMA Psychiat. 72, 882–891.
Beckmann, C.F., Mackay, C.E., Filippini, N., Smith, S.M., 2009. Group Comparison of Resting-State FMRI Data Using Multi-Subject ICA and Dual Regression. OHBM.
Behzadi, Y., Restom, K., Liau, J., Liu, T.T., 2007. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. NeuroImage 37, 90–101.
Bell, P.T., Shine, J.M., 2016. Subcortical contributions to large-scale network communication. Neurosci. Biobehav. Rev. 71, 313–322.
Buckner, R.L., Andrews-Hanna, J.R., Schacter, D.L., 2008. The brain's default network: anatomy, function, and relevance to disease. Ann. N. Y. Acad. Sci. 1124, 1–38.
Carhart-Harris, R.L., Leech, R., Erritzoe, D., Williams, T.M., Stone, J.M., Evans, J., Sharp, D.J., Feilding, A., Wise, R.G., Nutt, D.J., 2013. Functional connectivity measures after psilocybin inform a novel hypothesis of early psychosis. Schizophr. Bull. 39, 1343–1351.
Carhart-Harris, R.L., Kaelen, M., Bolstridge, M., Williams, T.M., Williams, L.T., Underwood, R., Feilding, A., Nutt, D.J., 2016a. The paradoxical psychological effects of lysergic acid diethylamide (LSD). Psychol. Med. 46, 1379–1390.
Carhart-Harris, R.L., Muthukumaraswamy, S., Roseman, L., Kaelen, M., Droog, W., Murphy, K., Tagliazucchi, E., Schenberg, E.E., Nest, T., Orban, C., Leech, R., Williams, L.T., Williams, T.M., Bolstridge, M., Sessa, B., McGonigle, J., Sereno, M.I., Nichols, D., Hellyer, P.J., Hobden, P., Evans, J., Singh, K.D., Wise, R.G., Curran, H.V., Feilding, A., Nutt, D.J., 2016b. Neural correlates of the LSD experience revealed by multimodal neuroimaging. Proc. Natl. Acad. Sci. U. S. A. 113, 4853–4858.
Cheng, W., Palaniyappan, L., Li, M., Kendrick, K.M., Zhang, J., Luo, Q., Liu, Z., Yu, R., Deng, W., Wang, Q., Ma, X., Guo, W., Francis, S., Liddle, P., Mayer, A.R., Schumann, G., Li, T., Feng, J., 2015. Voxel-based, brain-wide association study of aberrant functional connectivity in schizophrenia implicates thalamocortical circuitry. NPJ Schizophr. 1, 15016.
Choi, E.Y., Yeo, B.T., Buckner, R.L., 2012. The organization of the human striatum estimated by intrinsic functional connectivity. J. Neurophysiol. 108, 2242–2263.
Crossley, N.A., Mechelli, A., Scott, J., Carletti, F., Fox, P.T., McGuire, P., Bullmore, E.T., 2014. The hubs of the human connectome are generally implicated in the anatomy of brain disorders. Brain 137, 2382–2395.
De Gregorio, D., Comai, S., Posa, L., Gobbi, G., 2016. D-lysergic acid diethylamide (LSD) as a model of psychosis: mechanism of action and pharmacology. Int. J. Mol. Sci. 17.
Dittrich, A., 1998. The standardized psychometric assessment of altered states of consciousness (ASCs) in humans. Pharmacopsychiatry 31 (Suppl. 2), 80–84.
Dolder, P.C., Liechti, M.E., Rentsch, K.M., 2017a. Development and validation of an LC-MS/MS method to quantify LSD, iso-LSD, 2-oxo-3-hydroxy LSD, and nor-LSD and identify novel metabolites in plasma samples in a controlled clinical trial. J. Clin. Lab. Anal. 407, 1577–1584.
Dolder, P.C., Schmid, Y., Steuer, A.E., Kraemer, T., Rentsch, K.M., Hammann, F., Liechti, M.E., 2017b. Pharmacokinetics and Pharmacodynamics of Lysergic Acid Diethylamide in Healthy Subjects. Clin. Pharmacokinet. 56, 1219–1230.
Ferrarelli, F., Tononi, G., 2011. The thalamic reticular nucleus and schizophrenia. Schizophr. Bull. 37, 306–315.
Friston, K.J., 2011. Functional and effective connectivity: a review. Brain Connect. 1, 13–36.
Geyer, M.A., Vollenweider, F.X., 2008. Serotonin research: contributions to understanding psychoses. Trends Pharmacol. Sci. 29, 445–453.
Giraldo-Chica, M., Woodward, N.D., 2017. Review of thalamocortical resting-state fMRI studies in schizophrenia. Schizophr. Res. 180, 58–63.
Gouzoulis-Mayfrank, E., Habermeyer, E., Hermle, L., Steinmeyer, A., Kunert, H., Sass, H., 1998. Hallucinogenic drug induced states resemble acute endogenous psychoses: results of an empirical study. Eur. Psychiatry 13, 399–406.

Hale, J.R., Mayhew, S.D., Mullinger, K.J., Wilson, R.S., Arvanitis, T.N., Francis, S.T., Bagshaw, A.P., 2015. Comparison of functional thalamic segmentation from seed-based analysis and ICA. NeuroImage 114, 448–465.
Hollister, L.E., Hartman, A.M., 1962. Mescaline, lysergic acid diethylamide and psilocybin comparison of clinical syndromes, effects on color perception and biochemical measures. Compr. Psychiatry 3, 235–242.
Hollister, L.E., Sjoberg, B.M., 1964. Clinical syndromes and biochemical alterations following mescaline, lysergic acid diethylamide, psilocybin and a combination of the three psychotomimetic drugs. Compr. Psychiatry 5, 170–178.
Johansen, P., Krebs, T.S., 2015. Psychedelics not linked to mental health problems or suicidal behavior: a population study. J. Psychopharmacol. 29, 270–279.
Jones, E., 2007. The Thalamus. Cambridge University Press, New York.
Kelly Jr., R.E., Alexopoulos, G.S., Wang, Z., Gunning, F.M., Murphy, C.F., Morimoto, S.S., Kanellopoulos, D., Jia, Z., Lim, K.O., Hoptman, M.J., 2010. Visual inspection of independent components: defining a procedure for artifact removal from fMRI data. J. Neurosci. Methods 189, 233–245.
Khalili-Mahani, N., Chang, C., Van Osch, M.J., Veer, I.M., Van Buchem, M.A., Dahan, A., Beckmann, C.F., Van Gerven, J.M., Rombouts, S.A., 2013. The impact of "physiological correction" on functional connectivity analysis of pharmacological resting state fMRI. NeuroImage 65, 499–510.
Klaassens, B.L., Van Gorsel, H.C., Khalili-Mahani, N., Van Der Grond, J., Wyman, B.T., Whitcher, B., Rombouts, S.A., Van Gerven, J.M., 2015. Single-dose serotonergic stimulation shows widespread effects on functional brain connectivity. NeuroImage 122, 440–450.
Liechti, M.E., 2017. Modern clinical research on LSD. Neuropsychopharmacology 42, 2114–2127.
Liechti, M.E., Dolder, P.C., Schmid, Y., 2016. Alterations of consciousness and mystical-type experiences after acute LSD in humans. Psychopharmacology 234, 1499–1510.
Lisman, J.E., Pi, H.J., Zhang, Y., Otmakhova, N.A., 2010. A thalamo-hippocampal-ventral tegmental area loop may produce the positive feedback that underlies the psychotic break in schizophrenia. Biol. Psychiatry 68, 17–24.
Liu, T.T., 2013. Neurovascular factors in resting-state functional MRI. NeuroImage 80, 339–348.
Müller, F., Lenz, C., Dolder, P., Lang, U., Schmidt, A., Liechti, M., Borgwardt, S., 2017. Increased thalamic resting-state connectivity as a core driver of LSD-induced hallucinations. Acta Psychiatrica Scandinavica 136, 648–657.
Nichols, D.E., 2016. Psychedelics. Pharmacol. Rev. 68, 264–355.
Nichols, D.E., Johnson, M.W., Nichols, C.D., 2016. Psychedelics as medicines: an emerging new paradigm. Clin. Pharmacol. Ther. 68, 264–355.
Palhano-Fontes, F., Andrade, K.C., Tofoli, L.F., Santos, A.C., Crippa, J.A., Hallak, J.E., Ribeiro, S., De Araujo, D.B., 2015. The psychedelic state induced by ayahuasca modulates the activity and connectivity of the default mode network. PLoS One 10, e0118143.
Parent, A., Hazrati, L.N., 1995. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res. Brain Res. Rev. 20, 91–127.
Pinault, D., 2011. Dysfunctional thalamus-related networks in schizophrenia. Schizophr. Bull. 37, 238–243.
Power, J.D., Mitra, A., Laumann, T.O., Snyder, A.Z., Schlaggar, B.L., Petersen, S.E., 2014. Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage 84, 320–341.
Preller, K.H., Herdener, M., Pokorny, T., Planzer, A., Kraehenmann, R., Stampfli, P., Liechti, M.E., Seifritz, E., Vollenweider, F.X., 2017. The fabric of meaning and subjective effects in LSD-induced states depend on serotonin 2A receptor activation. Curr. Biol. 27, 451–457.
Raichle, M.E., Macleod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc. Natl. Acad. Sci. U. S. A. 98, 676–682.
Ray, K.L., McKay, D.R., Fox, P.M., Riedel, M.C., Uecker, A.M., Beckmann, C.F., Smith, S.M., Fox, P.T., Laird, A.R., 2013. ICA model order selection of task co-activation networks. Front. Neurosci. 7, 237.
Rickli, A., Moning, O.D., Hoener, M.C., Liechti, M.E., 2016. Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. Eur. Neuropsychopharmacol. 26, 1327–1337.
Roseman, L., Leech, R., Feilding, A., Nutt, D.J., Carhart-Harris, R.L., 2014. The effects of psilocybin and MDMA on between-network resting state functional connectivity in healthy volunteers. Front. Hum. Neurosci. 8, 204.
Schmid, Y., Enzler, F., Gasser, P., Grouzmann, E., Preller, K.H., Vollenweider, F.X., Brenneisen, R., Müller, F., Borgwardt, S., Liechti, M.E., 2015. Acute effects of lysergic acid diethylamide in healthy subjects. Biol. Psychiatry 78, 544–553.
Schmidt, A., Crossley, N.A., Harrisberger, F., Smieskova, R., Lenz, C., Riecher-Rossler, A., Lang, U.E., McGuire, P., Fusar-Poli, P., Borgwardt, S., 2016. Structural network disorganization in subjects at clinical high risk for psychosis. Schizophr. Bull. 43, 583–591.
Smith, S.M., Fox, P.T., Miller, K.L., Glahn, D.C., Fox, P.M., MacKay, C.E., Filippini, N., Watkins, K.E., Toro, R., Laird, A.R., Beckmann, C.F., 2009. Correspondence of the brain's functional architecture during activation and rest. Proc. Natl. Acad. Sci. U. S. A. 106, 13040–13045.
Spain, A., Howarth, C., Khrapitchev, A.A., Sharp, T., Sibson, N.R., Mar tin, C., 2015. Neurovascular and neuroimaging effects of the hallucinogenic serotonin receptor agonist psilocin in the rat brain. Neuropharmacology 99, 210–220.
Studerus, E., Gamma, A., Vollenweider, F.X., 2010. Psychometric evaluation of the altered states of consciousness rating scale (OAV). PLoS One 5, e12412.
Studerus, E., Gamma, A., Kometer, M., Vollenweider, F.X., 2012. Prediction of psilocybin response in healthy volunteers. PLoS One 7, e30800.
Tagliazucchi, E., Roseman, L., Kaelen, M., Orban, C., Muthukumaraswamy, S.D., Murphy, K., Laufs, H., Leech, R., McGonigle, J., Crossley, N., Bullmore, E., Williams, T., Bolstridge, M., Feilding, A., Nutt, D.J., Carhart-Harris, R., 2016. Increased global functional connectivity correlates with LSD-induced ego dissolution. Curr. Biol. 26, 1043–1050.
van den Heuvel, M.P., Sporns, O., 2011. Rich-club organization of the human connectome. J. Neurosci. 31, 15775–15786.
van den Heuvel, M.P., Sporns, O., 2013. Network hubs in the human brain. Trends Cogn. Sci. 17, 683–696.
Vardy, M.M., Kay, S.R., 1983. LSD psychosis or LSD-induced schizophrenia? A multimethod inquiry. Arch. Gen. Psychiatry 40, 877–883.
Vollenweider, F.X., Vollenweider-Scherpenhuyzen, M.F., Babler, A., Vogel, H., Hell, D., 1998. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 9, 3897–3902.
Whitfield-Gabrieli, S., Nieto-Castanon, A., 2012. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect. 2C, 125–141.
Yuan, R., Di, X., Taylor, P.A., Gohel, S., Tsai, Y.H., Biswal, B.B., 2016. Functional topography of the thalamocortical system in human. Brain Struct. Funct. 221, 1971–1984.
Zhang, D., Snyder, A.Z., Fox, M.D., Sansbury, M.W., Shimony, J.S., Raichle, M.E., 2008. Intrinsic functional relations between human cerebral cortex and thalamus. J. Neurophysiol. 100, 1740–1748.
Zhang, D., Snyder, A.Z., Shimony, J.S., Fox, M.D., Raichle, M.E., 2010. Noninvasive functional and structural connectivity mapping of the human thalamocortical system. Cereb. Cortex 20, 1187–1194.